Coupling of the glucagon receptor to adenylyl cyclase by GDP: Evidence for two levels of regulation of adenylyl cyclase*

(rat liver plasma membranes/GTPase/guanosine 5′-[β,γ-imido]triphosphate/adenylate cyclase)

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ABSTRACT   In rat liver plasma membranes preactivated with guanosine 5′-[β,γ-imido]triphosphate (GuoPP[NH]P), GDP promoted coupling of occupied glucagon receptor to adenylyl cyclase [adenylate cyclase; ATP, pyrophosphate-lyase (cyclizing), EC 4.6.1.1] with an apparent association constant K_a of 0.1–0.15 μM. The apparent K_a for the same effect of GTP was 0.2 μM. The effect of GDP was shown not to be due to GTP formed by putative transphosphorylation reaction(s) when ATP was present in the assay as substrate. In membranes not preactivated with GuoPP[NH]P, GDP both competitively inhibited GuoPP[NH]P stimulation of adenylyl cyclase (K_i 0.10 μM) and supported stimulation of cyclizing activity (apparent K_a 0.10 μM) by glucagon. These effects of GDP occurred in the absence of added GTP and in the absence of sufficient formation of GTP by putative transphosphorylation reaction(s) to account for them. It is concluded that two levels of regulation of liver adenylyl cyclase (cyclizing) activity must exit. One level is termed "receptor regulation"; it depends on occupancy of a receptor-related R site by nucleotide and is specific for either GDP or GTP. The second level of regulation is termed "GTPase regulation"; it is inhibited by GDP, depends on both GTP and GTPase, and accounts for activation of cyclizing activity by nonhydrolyzable analogs of GTP. The data suggest that both levels of regulation coexist and may synergize, one mediating responses to stimuli external to the cell (receptor regulation) and the other mediating stimuli of intracellular origin (GTPase regulation).

Adenylyl cyclases [adenylate cyclase; ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] are complex enzyme systems that appear to be composed of two dissimilar types of subunits, each having a separate catalytic activity: (*i*) a C subunit, or adenylyl cyclase proper, bearing the catalytic site for the cyclizing reaction and converting ATP to adenosine 3′,5′-monophosphate (cAMP) plus PP$_i$, and (*ii*) a G subunit or GTPase bearing a guanyl nucleotide binding site (G site) and catalyzing the hydrolysis of GTP to GDP plus P$_i$ (1–9). Optimal expression of cyclizing activity has been shown to depend on the presence of and the interaction between both subunits (3, 6, 10). According to genetic (3, 6, 10), biochemical (2, 7–9), and kinetic (11) studies, the enzyme system appears to exist in at least two states of cyclizing (and possibly also GTPase) activity: one with very little activity (basal activity) and the other active, the active state(s) being induced (stabilized) by GTP. Due to presence of the GTPase, the basic adenylyl cyclase system can be assumed to be in constant turnover (12).

The G site, as its name indicates, is specific for guanyl nucleotides. It interacts not only with GTP but also with GDP and GTP analogs such as guanosine 5′-[β,γ-imido]triphosphate (GuoPP[NH]P) (13) and guanosine 5′-[γ-thio]triphosphate (GTP[S]) (14, 15). In the presence of either [GuoPP[NH]P or GDP, the system's turnover cycle is blocked in two differing states of activity. Thus, GuoPP[NH]P leads to enhanced cy-

clizing activity, for the analog appears to mimic the action of GTP and, because it cannot be hydrolyzed by GTPase due to the β,γ-imido linkage, causes accumulation of the system as an enzyme–GuoPP[NH]P complex. Excess GDP, on the other hand, leads to a system with very low cyclizing activity (basal activity), for it appears that the GDP causes accumulation of the system as an enzyme–GDP complex with low activity not allowing the formation of enzyme–GTP and the ensuing activation of the cyclization reaction. Evidence both from Levinson and Blume (16) using neuroblastoma cells and more recently from Cassel and Selinger (17) using turkey erythrocytes suggests that the affinity and the release rate of GDP from the system may be the limiting steps regulating the system's rate of turnover—i.e., the rate at which, under the influence of GTP, the reaction sequence free enzyme → enzyme–GTP → enzyme–GDP → free enzyme occurs. Furthermore, work by the same authors suggests that receptor stimulation of cyclizing activity may be merely the result of a stimulation of the GDP release rate. Thus, Levinson and Blume (16) demonstrated that hormone treatment of neuroblastoma cell adenylyl cyclase decreases the affinity of the system for inhibitory GDP (tested against GuoPP[NH]P), and Cassel and Selinger (17) showed that hormone treatment of turkey erythrocyte membranes preloaded with [^{3}H]GDP results in rapid release of the label. Thus, the concept has emerged that positive coupling of hormone–receptor complex to the adenylyl cyclase system (i.e., stimulation of cyclizing activity) involves the dissociation of GDP from the inactive state of the system followed by binding of GTP and activation of ATP to cyclic AMP conversion (16, 17). Therefore, GTP and its associated GTPase appear to play an obligatory and central role in receptor regulation of the basic two-subunit adenylyl cyclase system.

It might then be envisioned that, in the absence of the GTP and GTPase-driven turnover of the system, hormonal regulation of enzymatic activity cannot occur. While this supposition appears to hold true in many instances in which the system is fully activated either due to binding of a ligand not susceptible to hydrolysis by GTPase [e.g., GuoPP[NH]P or GTP[S] (4, 5, 11, 12, 15, 18, 19)], or due to the inhibition of the GTPase itself [e.g., ADP-ribosylation by cholera toxin (6–8), or intracellular factors (20)], experiments testing for the effects of GDP on hormonal stimulation of the glucagon-sensitive adenylyl cyclase system indicate otherwise. The present report presents some of our experiments on the capacity of GDP to promote positive coupling of glucagon receptor complex to the adenylyl cyclase system in the absence of measurably significant concentrations of GTP.

Abbreviations: GuoPP[NH]P, guanosine 5′-[β,γ-imido]triphosphate; AdoPP[NH]P, adenosine 5′-[β,γ-imido]triphosphate; GTP[S], guanosine 5′-[γ-thio]triphosphate; cAMP, adenosine 3′,5′-monophosphate.
* This is the second paper in a series exploring the characteristics of hormone receptor coupling to adenylyl cyclase. The first paper is ref. 22.

EXPERIMENTAL PROCEDURES

Materials. [α-^{32}P]GTP and [α-^{32}P]ATP were purchased from International Chemical and Nuclear Corporation or prepared in our laboratory as described (21). [α-^{32}P]GDP was prepared by acid hydrolysis (pH 2) of [α-^{32}P]GTP. The [α-^{32}P]GDP was separated from [^{32}P]GMP and [α-^{32}P]GTP by chromatography on DEAE-Sephadex (21). Unlabeled GDP was obtained from Sigma (catalog number 6506) and was found to contain less than 1% GTP as assessed by chromatography on DEAE-Sephadex (21). Plastic-backed polyethyleneimine-cellulose thin-layer sheets were from Brinkmann. The sources of all other materials used throughout are those described (22, 23).

Adenylyl Cyclase Assay. Conditions of assay included [α-^{32}P]ATP (50–100 Ci/mmol; 1 Ci = 3.7 × 10^{10} becquerels), 0.5 mM ATP or 0.5 mM adenosine 5'-[β,γ-imido]triphosphate (Ado*PP*[NH]*P*), 5.0 mM MgCl$_2$, 1.0 mM EDTA, 1.0 mM [^{3}H]cAMP (approximately 10,000 cpm per assay), 25 mM 1,3-bis[tris(hydroxymethyl)methylamino]propane·HCl (Sigma) at pH 7.5, and partially purified liver plasma membranes (24) at 0.01–0.03 mg of protein per ml—i.e., 0.5–1.5 μg of membrane protein per 50-μl assay. Incubations were for 5 min at 32.5°C, after which time they were stopped (22) and [^{32}P]cAMP formed was assayed according to Salomon *et al.* (25) as modified by Bockaert *et al.* (26).

Purification Procedures. ATP used throughout was subjected to column chromatography to separate contaminating guanyl nucleotide-like components as described (23). While it was not established that indeed all guanyl nucleotide-like contamination was removed, the procedure used was judged satisfactory in view of the fact that it allowed detection of effects of GTP and GDP omission and readdition that were otherwise not evident.

Procedure for Preactivation by Guo*PP*[NH]*P* Followed by Washing. The procedure described recently (22) was used. Briefly, liver membranes (0.2 mg/ml) were incubated at 32.5°C for 30 min in the presence of 0.1 mM ATP under adenylyl cyclase assay conditions except that [α-^{32}P]ATP and [^{3}H]cAMP were omitted. When present, Guo*PP*[NH]*P* was 0.1 mM. At the end of the incubation the mixture was diluted 1:5 with a solution containing 25 mM Tris·HCl, 1 mM EDTA at pH 7.5, 5 mM MgCl$_2$, 1 mM cAMP, 0.5 mM ATP, and 10 μM GTP, incubated 10 min at 32.5°C, and then centrifuged at 4°C for 15 min at 10,000 × *g*. The supernatant was discarded and the pellet was resuspended in 25 mM Tris·HCl, pH 7.5, with the aid of a syringe fitted with a 22-gauge needle. The centrifugation and resuspension procedure was repeated twice more and the final membrane pellet was suspended in 25 mM Tris·HCl, pH 7.5, to give between 0.08 and 0.1 mg of membrane protein per ml and yielding "preactivated and washed membranes." Recovery of protein was routinely between 40 and 50%. Preactivation by Guo*PP*[NH]*P* followed by washing under these conditions results in maintenance of more than 95% of the Guo*PP*[NH]*P*-activated state of adenylyl cyclase system, as assessed by lack of stimulability of Guo*PP*[NH]*P* of the enzyme system in the washed membrane (22).

Estimation of GTP and GDP Levels at the End of Incubations. The procedure described elsewhere for estimation of maintenance of ATP levels (27) was used. Briefly, adenylyl cyclase assay incubations were repeated under identical conditions but omitting [α-^{32}P]ATP and adding the equivalent of 10^6 cpm per assay of [α-^{32}P]GTP or [α-^{32}P]GDP. The reactions were stopped by addition of 100 μl of 10.0 mM GTP/10.0 mM GDP/10.0 mM GMP/1% sodium dodecyl sulfate and immediate cooling to 0–4°C. Distribution of radioactivity into material chromatographing with R_F values of GTP and GDP was

evaluated by seeding small aliquots (1–2 μl) of the stopped reaction mixtures on plastic-covered thin-layer sheets of polyethyleneimine cellulose, chromatographing (ascending) with 1 M LiCl as developing solvent, and determining the distribution of radioactivity by liquid scintillation counting.

Protein was determined by the Lowry method (28) with bovine serum albumin as standard.

RESULTS

Experiments from several laboratories (28, 29, 19), including our own (30, 22), indicate that two separate nucleotide sites are involved in hormonal stimulation of adenylyl cyclases such as the glucagon-sensitive one from liver membranes. One site is the G site on the G subunit of the basic system referred to in the introduction. The second site is a receptor-related site (R site) responsible for regulation of receptor behavior—i.e., binding affinity (31), binding rates (31), and coupling (22).[†] Because the original work on nucleotide effects on binding of glucagon to liver membranes had shown GDP to be as good a ligand as GTP in promoting dissociation of ^{125}I-labeled glucagon from its specific binding sites under conditions in which no transphosphorylation was possible, it became of interest to determine whether this nucleotide, capable of altering binding behavior, would act either agonistically or antagonistically in a coupling assay such as the one recently developed in our laboratory (22). To this end, liver membrane adenylyl cyclase, preactivated with Guo*PP*[NH]*P* and washed free of unbound nucleotides, was exposed to saturating concentrations of glucagon and various concentrations of GDP and then assayed for cyclizing activity under two conditions, both lacking a nucleotide triphosphate-regenerating system yet allowing for preservation of [α-^{32}P]ATP used as substrate: (*i*) the assay medium contained only [α-^{32}P]ATP (0.5 mM, specific activity 800–1000 cpm/pmol), or (*ii*) the assay medium contained 0.5 mM of the synthetic ATP analog Ado*PP*[NH]*P* (this latter condition, while artificial, was used to preclude the possibility that some of the effects seen were due to the presence of a putative ATP-dependent transphosphorylation system), and 2 × 10^7 cpm of [α-^{32}P]ATP per 50-μl assay. Low concentrations of membranes (0.5–1.0 μg of membrane protein per 50-μl assay) were also used to minimize membrane-mediated nucleotide interconversion reactions. Fig. 1 shows that GDP is a potent (apparent association constant, K_a, 0.1–0.15 μM) agonist in stimulating coupling of glucagon receptor to the liver adenylyl cyclase system. The possibility existed that the actual mediator was GTP formed by a transphosphorylation reaction, rather than GDP. The following findings rule out this possibility. As shown in Fig. 1, the levels of GDP did not change significantly at any of the concentrations used and only negligible proportions of the added GDP cochromatographed in the place of GTP. Assuming that radioactivity migrating with an R_F of GTP indeed represented GTP, then at the concentration of GDP that gave 50% of maximum stimulation of coupling (0.15 μM) a maximum of 0.00075 μM GTP was present. Fig. 2 presents the same experiments done with GTP as the stimulating nucleotide and shows that 0.2 μM GTP is necessary for 50% of maximum stimulation of coupling. Thus, the effect of 0.15 μM GDP seen in the experiments of Fig. 1 cannot be ascribed to GTP formed during the assay. These experiments indicate that both GTP and GDP

[†] Although nucleotides affect two separate receptor-related functions (binding to hormone and coupling to adenylyl cyclase) and it is possible that each functional change is mediated by a separate site, we assume for the sake of simplicity that a single guanyl nucleotide-specific site on or near the receptor (R site) mediates both effects of nucleotides.

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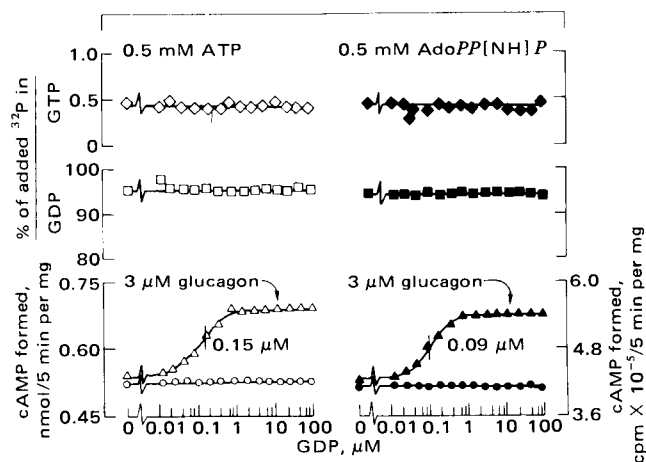Proc. Natl. Acad. Sci. USA 76 (1979)   3191

FIG. 1.   Stimulation of receptor coupling to a GuoPP[NH]P preactivated and washed adenylyl cyclase system by GDP; lack of GDP-derived formation of GTP. Membranes were preactivated in the presence of GuoPP[NH]P, washed, and then incubated with various concentrations of GDP in the absence (O, ●) and in the presence (△, ▲) of 3 μM glucagon. (Left) Assays performed with 0.5 mM ATP in the medium; (Right) assays performed with 0.5 mM AdoPP[NH]P in the medium; (Bottom) adenylyl cyclase activities; (Middle) percentage of total [α-^{32}P]GDP added migrating upon thin-layer chromatography as GDP at the end of the incubations; and (Top) percentage of total [α-^{32}P]GDP added migrating as GTP at the end of the incubations. The assay contained 5.0 mM MgCl$_2$, 1 mM EDTA, and no nucleoside triphosphate-regenerating system. K_a values for GDP are shown by vertical lines in Bottom.

FIG. 3.   Competitive inhibition of GuoPP[NH]P stimulation of basal adenylyl cyclase activity by GDP. Assays were carried out for 5 min in the presence of 0.5 mM AdoPP[NH]P, 5 mM MgCl$_2$, 1 mM EDTA, and the indicated concentrations of GDP. No nucleoside triphosphate-regenerating system was used. O, No GuoPP[NH]P; △, 0.5 μM GuoPP[NH]P; ▲, 10 μM GuoPP[NH]P. IC$_{50}$, concentration of inhibitor required to achieve 50% of maximally obtained inhibition.

We investigated whether any significant differences could be detected between the apparent K_a of the R site for GDP and the affinity of the G site for GDP, assessed by competitive inhibition of GuoPP[NH]P stimulation of control (not preactivated) membrane adenylyl cyclase in the absence of glucagon. As shown in Fig. 3, GDP inhibited competitively GuoPP[NH]P-mediated activation of the liver adenylyl cyclase

are equally effective in promoting coupling of glucagon receptor to the adenylyl cyclase system and that, therefore, the R site does not distinguish between GTP and GDP. As indicated by the fact that 0.5 mM ATP did not promote coupling (guanyl nucleotide addition was still necessary), the R site of the liver glucagon receptor is highly specific for guanyl nucleotides.
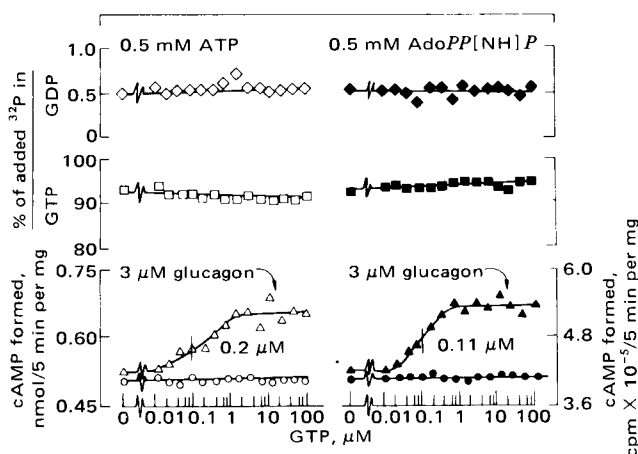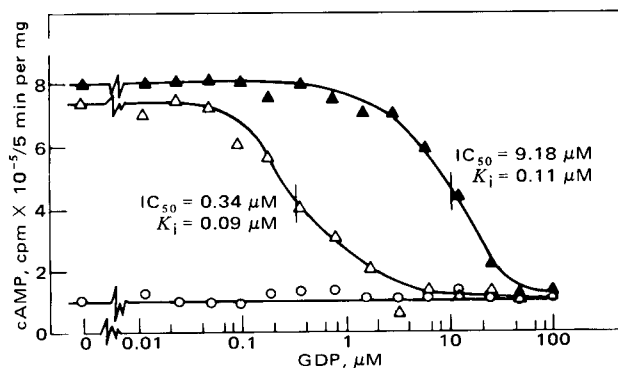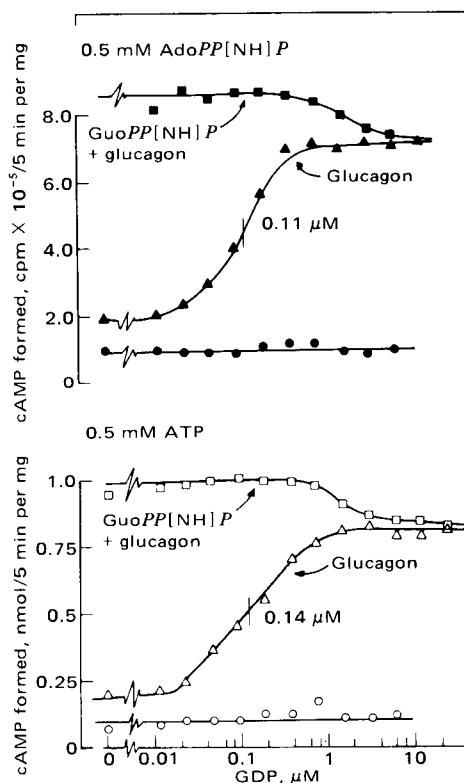

FIG. 2.   Stimulation of receptor coupling to a GuoPP[NH]P preactivated and washed adenylyl cyclase system by GTP; effect of incubation on maintenance of GTP levels and appearance of GDP in the incubation medium. Experimental conditions were the same as for the experiments described in the legend to Fig. 1 except that GDP was omitted and various concentrations of GTP were added. (Bottom) Assays performed with [α-^{32}P]ATP to monitor cAMP formation; (Middle and Top) assays performed with [α-^{32}P]GTP to monitor distribution of radioactivity into GTP (Middle) and GDP (Top). Incubations in the presence of 3 μM glucagon are indicated (△, ▲). The assay contained 5.0 mM MgCl$_2$, 1 mM EDTA, and no nucleoside triphosphate-regenerating system.

FIG. 4.   Effect of varying the concentration of GDP on cyclizing activity in the absence (O, ●) and in the presence of 3 μM glucagon (△, ▲) or 3 μM glucagon plus 10 μM GuoPP[NH]P (□, ■). (Lower) Assays performed in the presence of 0.5 mM ATP; (Upper) assays performed in the presence of 0.5 mM AdoPP[NH]P. Assays contained 5 mM MgCl$_2$ 1 mM EDTA, and no nucleoside triphosphate-regenerating system. Apparent K_a values for GDP are shown by vertical lines.
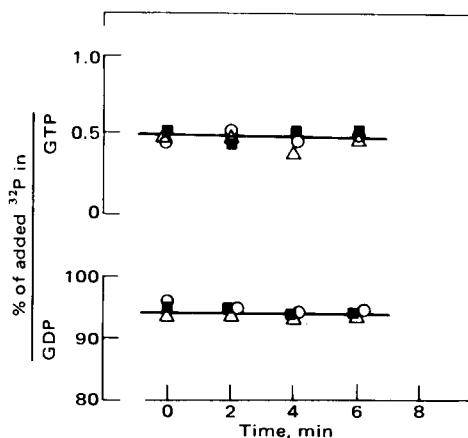
FIG. 5.   Lack of effect of glucagon on formation of GTP from GDP. Liver membranes (0.92 μg of protein per assay) were incubated for 2, 4, or 6 min at 32.5°C under the adenylyl cyclase assay conditions used throughout the present studies, including 0.5 mM ATP and 10 μM [α-^{32}P]GDP (0.96 × 10^7 cpm/50-μl assay) and omitting [α-^{32}P]ATP and a nucleoside triphosphate-regenerating system. At the indicated times the incubations were stopped and the distribution of label between GDP and GTP was determined. O, No membranes; Δ, membranes; ■, membranes plus 3 μM glucagon.

with a K_i of 0.1 μM. We attempted to determine whether this value would be altered by hormonal stimulation of the system, as suggested by the work of Levinson and Blume (16) in the neuroblastoma adenylyl cyclase system and found (Fig. 4) that GDP did not substantially inhibit GuoPP[NH]P-enhanced glucagon-stimulated cyclizing activity. Moreover, GDP promoted glucagon-stimulated enhancement of cyclizing activity in the absence of GuoPP[NH]P, under conditions in which the G site of the system was undoubtedly occupied by GDP (Fig. 4). Fig. 5 shows in an assay with 0.92 μg of membrane protein, 10 μM GDP, and 0.5 mM ATP that glucagon does not promote transphosphorylation of GDP to GTP. Hence, it appears that receptor-mediated enhancement of cyclizing activity of the liver plasma membrane adenylyl cyclase system does not require GTP. Although hormonal stimulations of adenylyl cyclases in the presence of GDP have been observed earlier (32–34), one study found GDP-to-GTP transphosphorylation to occur (33), and others did not determine whether GTP was being formed (32, 34).

DISCUSSION

The fact that true activation of cyclizing activity can be obtained in response to hormone in the absence of GTP and, hence, modulation of GTPase activity in a system that, like others, is activated by GuoPP[NH]P and cholera toxin (both conditions known to stop the turnover cycle in the so-called active "GTP-state" of the system) (11, 35) strongly suggests that adenylyl cyclase systems are subject to two levels of regulation, which can coexist and synergize or proceed in relative independence from each other. The better known of the two levels of regulation is the one pertaining to hormone receptor action. It involves the obligatory participation of one nucleotide site associated with the receptor (R site) and possibly also of a second site (G site) on the cyclase system. Clearly, the sites are different, because it is possible to occupy the G site with one nucleotide (GuoPP[NH]P), and to promote coupling with another [GDP (Fig. 1) or GTP (Fig. 2)]. It is not clear, however, whether receptor-mediated stimulation of cyclizing activity, requiring guanyl nucleotide occupancy of the R site, also requires occupancy of the G site. Kinetic arguments to be presented else-

where (ref. 11; L. Birnbaumer, R. Iyengar, T. L. Swartz, and J. Abramowitz, unpublished) argue that, if glucagon receptor-mediated activation of cyclizing activity can occur upon occupancy of the G site by both GTP and GDP, there is no need for this site at all, provided only two states of enzyme activity are involved—one with little or no activity, and the other active and stabilized by GTP in the absence of hormone and stabilized by active hormone receptor regardless of nucleotide occupancy. It may be concluded that this level of regulation, which we may term "receptor regulation," is, as its name indicates, primarily responsible for changes in cAMP levels that occur in response to hormonal stimuli coming from without the cells.

The second level of regulation is less well understood, mainly because of its relatively recent discovery (4, 5). It involves both GTP and the GTPase of the G component of the adenylyl cyclase system as obligatory partners. We would like to term this type of regulation "GTPase regulation." This type of regulation does not preclude receptor regulation. It depends on three factors: GTP levels, GDP levels [which in the absence of hormones lead to inhibition of cyclizing activity (Fig. 3)], and GTPase activity. The last is subject to reversible inhibition by ADP-ribosylation due to extracellular toxins such as cholera toxin (5, 7–9) or to intracellular ADP-ribosylating factors such as the one recently purified by Moss and Vaughan (20) from the cytosol of turkey erythrocytes. The possibility should be entertained that changes in cAMP levels occurring throughout the cell cycle (for review, see ref. 36) without obvious variations in external medium composition may be due to changing ADP-ribosylating activities altering cyclizing activity via GTPase regulation. Of importance is that neither type of regulation interferes with the other in the rat liver adenylyl cyclase inasmuch as a basal system can always be activated regardless of whether by receptor regulation, GTPase regulation, or both.

1.   Sutherland, E. W. & Rall, T. W. (1962) *J. Biol. Chem.* **237**, 1220–1227.
2.   Pfeuffer, T. (1977) *J. Biol. Chem.* **252**, 7224–7234.
3.   Ross, E. M. & Gilman, A. G. (1977) *J. Biol. Chem.* **252**, 6966–6969.
4.   Cassel, D. & Selinger, Z. (1976) *Biochim. Biophys. Acta* **452**, 538–551.
5.   Cassel, D. & Selinger, Z. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3307–3311.
6.   Johnson, G. L., Kaslow, H. R. & Bourne, H. R. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3113–3117.
7.   Cassel, D. & Pfeuffer, T. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2669–2673.
8.   Gill, D. M. & Meren, R. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3050–3054.
9.   Johnson, G. L., Kaslow, H. R. & Bourne, H. R. (1978) *J. Biol. Chem.* **253**, 7120–7123.
10.   Ross, E. M., Howlett, A. C., Ferguson, K. M. & Gilman, A. G. (1978) *J. Biol. Chem.* **253**, 6401–6412.
11.   Iyengar, R., Swartz, T. L., Abramowitz, J. & Birnbaumer, L. (1979) *J. Supramol. Struc.* **9**, 23 (abstr.).
12.   Cassel, D., Levkovitz, H. & Selinger, Z. (1977) *J. Cycl. Nucl. Res.* **3**, 393–406.
13.   Londos, C., Salomon, Y., Lin, M. C., Harwood, J. P., Schramm, M., Wolff, J. & Rodbell, M. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 3087–3090.
14.   Pfeuffer, T. & Helmreich, E. J. M. (1975) *J. Biol. Chem.* **250**, 867–876.
15.   Cassel, D. & Selinger, Z. (1977) *Biochem. Biophys. Res. Commun.* **77**, 868–873.

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Proc. Natl. Acad. Sci. USA 76 (1979) 3193

16. Levinson, S. L. & Blume, A. J. (1977) *J. Biol. Chem.* **252,** 3766–3774.
17. Cassel, D. & Selinger, Z. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 4115–4159.
18. Rendell, M. S., Rodbell, M. & Berman, M. (1977) *J. Biol. Chem.* **252,** 7909–7912.
19. Svoboda, M., Robberecht, P. & Christopher, J. (1978) *FEBS Lett.* **92,** 351–356.
20. Moss, J. & Vaughan, M. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 3621–3624.
21. Birnbaumer, L., Torres, H. N., Flawia, M. M. & Fricke, R. F. (1979) *Anal. Biochem.* **92,** 124–133.
22. Iyengar, R., Swartz, T. L. & Birnbaumer, L. (1979) *J. Biol. Chem.* **254,** 1119–1123.
23. Iyengar, R., Mintz, P. W. & Birnbaumer, L. (1979) *J. Biol. Chem.* **254,** in press.
24. Pohl, S. L., Birnbaumer, L. & Rodbell, M. (1971) *J. Biol. Chem.* **246,** 1849–1856.
25. Salomon, Y., Londos, C. & Rodbell, M. (1974) *Anal. Biochem.* **58,** 541–548.
26. Bockaert, J., Hunzicker-Dunn, M. & Birnbaumer, L. (1976) *J. Biol. Chem.* **251,** 2653–2663.

27. Birnbaumer, L., Yang, P.-C., Hunzicker-Dunn, M., Bockaert, J. & Duran, J. M. (1976) *Endocrinology* **99,** 163–184.
28. Welton, A. F., Lad, P. M., Newby, A. C., Yamamura, H., Nicosia, S. & Rodbell, M. (1977) *J. Biol. Chem.* **252,** 5947–5950.
29. Ross, E. M., Maguire, M. E., Sturgill, T. W., Biltonen, R. L. & Gilman, A. G. (1977) *J. Biol. Chem.* **252,** 5761–5775.
30. Birnbaumer, L. & Yang, P.-C. (1974) *J. Biol. Chem.* **249,** 7867–7873.
31. Rodbell, M., Krans, H. M. J., Pohl, S. L. & Birnbaumer, L. (1971) *J. Biol. Chem.* **246,** 1872–1876.
32. Hanoune, J., Lacombe, M. L. & Pecker, F. (1975) *J. Biol. Chem.* **250,** 4569–4574.
33. Clark, R. B. (1978) *J. Cyclic Nucl. Res.* **4,** 71–85.
34. Kimura, N. & Nagata, N. (1977) *J. Biol. Chem.* **252,** 3829–3835.
35. Lin, M. C., Welton, A. F. & Berman, M. F. (1978) *J. Cyclic Nucl. Res.* **4,** 159–168.
36. Friedman, D. L., Johnson, R. A. & Zeilig, C. A. (1977) in *Advances in Cyclic Nucleotide Research,* eds. Greengard, P. & Robison, A. (Raven, New York), Vol. 7, pp. 69–114.